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Ethanol synthesis based on the photoredox system consisting of photosensitizer and dehydrogenases

Yutaka Amao[a,b,c,*], Naho Shuto[d], Hideharu Iwakuni[e]

[a] Advanced Research Institute for Natural Science and Technology, Osaka City University, Sugimoto 3-3-138, Sumiyoshi-ku, Osaka 558-8585, Japan
[b] Research Centre for Artificial Photosynthesis (ReCAP), Osaka City University, Sugimoto 3-3-138, Sumiyoshi-ku, Osaka 558-8585, Japan
[c] Precursory Research for Embryonic Science and Technology (PRESTO), Japan Science and Technology Agency, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan
[d] Department of Applied Chemistry, Oita University, Dannoharu 700, Oita 870-1192, Japan
[e] Mazda Motor Corporation, 3-1 Shinchi, Fuchu-cho, Aki-gun, Hiroshima 730 867, Japan

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ABSTRACT

Photochemical synthesis of ethanol from acetate was investigated with the system aldehyde (AldDH) and alcohol (ADH) dehydrogenases from Yeast, and methylviologen (MV^{2+}) photoreduction by the visible light photosensitization of chlorophyll derivative, chlorin-e_6 of zinc complex (ZnChl-e_6) in the presence of NADPH as an electron donating reagent. Irradiation of a solution containing NADPH, ZnChl-e_6, MV^{2+}, AldDH, ADH and sodium acetate with visible light resulted in ethanol synthesis. The concentration of ethanol produced was 1.4 mM after 150 min irradiation under the condition of NADPH (3.3 mM), ZnChl-e_6 (100 μM), MV^{2+} (12 mM), AldDH (0.22 μM), ADH (6.7 nM) and sodium acetate (30 mM) in 50 mM of sodium pyrophosphate buffer pH 7.4).

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1. Introduction

Photoredox system consisting of an electron donor, a photosensitizer, an electron carrier and catalyst is widely used for the photochemical conversion system [1–5]. This system is a simplification of the photosynthesis reaction, thus, is used as a model of artificial photosynthesis. By using reduced form of electron carrier molecule as a substrate for the catalyst in this system, hydrogen production and carbon dioxide conversion systems would be developed. For photoinduced hydrogen production systems, platinum nano-particle and enzyme, hydrogenase from *Desulfovibrio vulgaris* (Miyazaki) have been widely used as catalysts [6–10]. The photochemical and enzymatic synthesis of organic compounds has also been developed using this system in the presence of various enzymes [11–22]. Dehydrogenase enzymes such as lactate (LDH), formate (FDH), aldehyde (AldDH) and alcohol dehydrogenase (ADH) are useful enzymes for the synthesis of valuable organic compounds such as lactic acid, formic acid, methanol etc. For example, we reported the lactic acid production with L-lactate dehydrogenase from Pig heart (LDH) and reduced methyl viologen (MV^{2+}) produced by the visible light photosensitization of water soluble zinc tetrakis(4-methylpyridyl) porphyrin (ZnTMPyP) and accomplishment of 17.0% yield of pyruvic acid to lactic acid. after 4 h irradiation [22]. The advantage of the combination of photoredox system and enzyme is that only the desired product will be obtained with the visible light energy.

In the photoredox system, the effective photosensitiser also is an essential component. Ruthenium(II) coordination compounds and porphyrins are widely used as effective photosensitizers [23,24]. As water soluble zinc porphyrins, zinc tetraphenylporphyrin tetrasulfonate (ZnTPPS) and zinc tetrakis (4-methylpyridyl) porphyrin (ZnTMPyP), have a strong absorption bands in the visible light region (380–600 nm), these porphyrins are more widely used as an effective photosensitiser than ruthenium(II) coordination compounds [25–28]. In contrast, photosynthesis dyes such as chlorophylls and its derivatives play important roles in light-harvesting, photoinduced energy transfer and charge separation in photosynthesis reactions. These dyes have the absorption maxima at 670–800 nm and the molar absorption coefficient of these dyes is larger than that of zinc porphyrin or ruthenium(II) coordination compound [29–31]. However, typical chlorophyll, Mg chlorophyll-*a* (Mg Chl-*a*) purified from green plant is unstable against the irradiation. On the other hand, zinc bacteriochlorophyll *a* (Zn BChl-*a*) was founded in an aerobic bacterium *Acidiphilium rubrum* [32]. As

* Corresponding author at: Advanced Research Institute for Natural Science and Technology, Osaka City University, Sugimoto 3-3-138, Sumiyoshi-ku, Osaka 558-8585, Japan. Fax: +81 6 6605 3726.
E-mail address: amao@ocarina.osaka-cu.ac.jp (Y. Amao).

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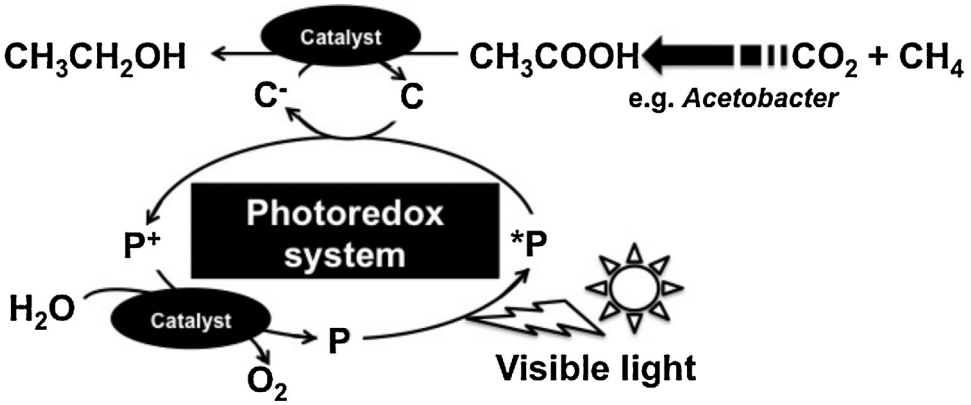

Fig. 1. Concept of visible-light induced ethanol synthesis from carbon dioxide, water and methane with the coupling system of photoredox, catalysts and biological materials.

the zinc porphyrins are stable against the irradiation and effective photosensitizers, zinc chlorophylls are attractive compound as a stable visible photosensitizer. Some studies on the preparation and characterization of the zinc chlorophyll and bacteriochlorophylls have been reported [33,34]. As chlorophylls and bacteriochlorophylls are insoluble to aqueous solution, chlorin-e_6 (Chl-e_6) formed by the hydrolysis of chlorophyll-a has three carboxylate groups in molecule. Zinc complex of Chl-e_6 (Zn Chl-e_6) also is attractive compound as an effective visible light photosensitizer with higher photostability for photoredox system.

Recently, ethanol is paid much attention to the lower carbon fuel. The largest single use of ethanol is as a motor fuel and fuel additive. Thus, we devoted to the synthesis of ethanol from carbon dioxide, natural gas such as methane and water with coupling the photoredox system, some catalysts and biological material as shown in Fig. 1. One of the important steps is the photochemical ethanol synthesis from acetate using combination of photoredox and catalyst system. By using natural co-enzyme nicotinamide adenine dinucleotide (NAD^+) or its' reduced form (NADH), AldDH catalyses both the reduction of acetate to acetaldehyde and the oxidation of acetaldehyde to acetate, and ADH also catalyses both the reduction of acetaldehyde to ethanol and the oxidation of ethanol to acetaldehyde as shown in Fig. 2. Thus, it is necessary to suppress the oxidation of acetaldehyde to acetate with AldDH and the oxidation of ethanol to acetaldehyde with ADH for the conversion of acetate to ethanol.

On the other hand, we found that the reduced form of MV^{2+} only acts as an artificial coenzyme for ADH and AldDH, and MV^{2+} is not recognized for artificial coenzyme of ADH and AldDH. Thus, the oxidation of acetaldehyde to acetate with AldDH and the oxidations of ethanol to acetaldehyde with ADH suppressed using MV^{2+} as an electron carrier as shown in Fig. 2. As MV^{2+} easily is reduced using photoredox system, moreover, MV^{2+} is suitable molecule for the photochemical ethanol synthesis from acetate with AldDH and ADH.

For water photolysis system, development of the optimal catalyst for oxidizing water especially attracts expectation. Among some catalysts for water photolysis, Chloroplast from green plant is attracting attention as an oxidation catalyst of water. Chloroplast has two important sites. One is the photosystem I with reaction center P700, acts as a photoreduction of $NADP^+$ to NADPH. The other one is the photosystem II with reaction center P680, acts as an oxygen production based on the water oxidation. By using the $NADP^+$/NADPH redox coupling, the combination system of water photolysis using Chloroplast (process 1) and the photochemical ethanol synthesis from acetic acid with ZnChl-e_6, MV^{2+}, AldDH, and ADH (process 2) will be developed as shown in Fig. 3.

Here we describe a development of the photochemical ethanol synthesis from acetic acid with AldDH and ADH, and reduced MV^{2+} produced by the visible light photosensitization of ZnChl-e_6 in the presence of NADPH as an electron donor in the process 2 of Fig. 3. The principle of this photoredox system is as follows. When the visible-light is irradiated to Zn Chl-e_6, Zn Chl-e_6 in photoexcited state (*Zn Chl-e_6) is formed. In the next step, reduced MV^{2+} ($MV^{\bullet+}$) is produced by the electron transfer from *Zn Chl-e_6 to MV^{2+}. Subsequently, $MV^{\bullet+}$ acts on AldDH and ADH as a co-enzyme, acetate is converted to ethanol via acetaldehyde. Finally, the electron transfers to the one-electron oxidized form of Zn Chl-e_6 (Zn Chl-e_6^+) from NADPH as an electron donor, Zn Chl-e_6 in the ground state is produced. In this paper, the investigation for the optimum condition of methanol synthesis from acetic acid using the system as shown in Fig. 2 also was studied.

2. Experimental

2.1. Materials

Chl-e_6 and sodium acetate, sodium pyrophosphate are purchased from Wako Pure Chemical Industries Ltd. Methylviologen dichloride (MV^{2+}) is supplied by Tokyo Kasei Co. Ltd. NADH and NADPH are purchased from Oriental Yeast Co. Ltd. AldDH from Yeast (molecular weight 200 kDa) is obtained from Roche Co. Ltd. ADH from Yeast (molecular weight 80 kDa) is purchased from Oriental Yeast Co. Ltd. The other chemicals are analytical grade or the highest grade available.

2.2. Preparation of Zn Chl-e_6

Zn Chl-e_6 was synthesized by refluxing Chl-e_6 with about 10 times the molar equivalent of zinc acetate in 100 ml methanol at 60 °C for 2 h according to the previously reported method [35–38]. The production of Zn Chl-e_6 was monitored by UV–vis absorption spectrum using Shimadzu Multispec 1500 spectrophotometer. The characteristic absorption bands of Zn Chl-e_6 at 418 and 638 nm increased and the absorbance at 400, 514 and 660 nm due to Chl-e_6 decreased gradually during reaction. After the mixture was cooled to room temperature and then the solvent was removed under vacuum. To remove the unreacted zinc acetate dehydrate, the reaction mixture was washed with water and Zn Chl-e_6 was precipitated in water. Zn Chl-e_6 was collected by filtration and washed with water and then the purification was performed by recrystallization from water-methanol (5:1) solution.

2.3. Photoreduction of MV^{2+}

A solution containing Zn Chl-e_6 (100 μM), MV^{2+}, and NADPH (3.3 mM) in 3.0 ml of 50 mM sodium pyrophosphate buffer (pH 7.4) was deaerated by freeze–pump–thaw cycles repeated 6 times. The

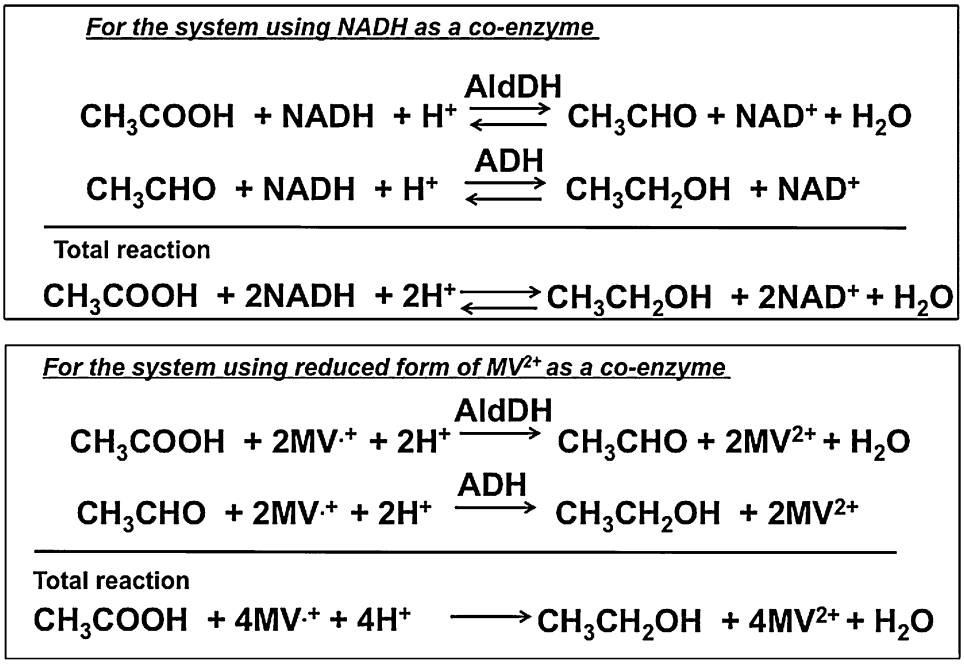

Fig. 2. Ethanol production from acetate scheme with AldDH and ADH using natural co-enzyme NAD^+/ NADH and artificial co-enzyme MV^{2+}/$MV^{\bullet+}$.

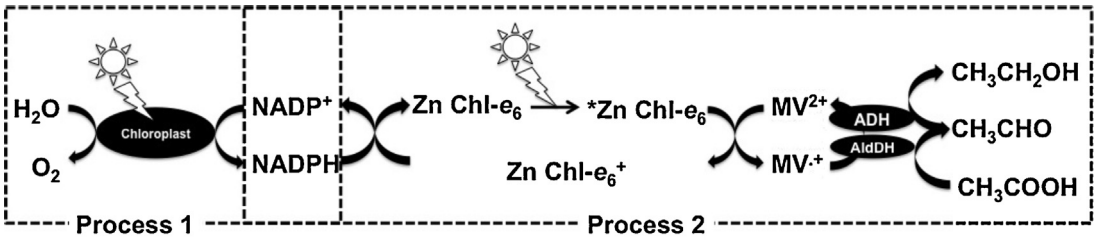

Fig. 3. The system combination of water photolysis using Chloroplast (process 1) and photochemical ethanol synthesis from acetate with the system consisting of Zn Chl-e_6, MV^{2+}, AldDH and ADH (process 2) via the $NADP^+$/ NADPH redox coupling.

sample solution was irradiated with a 250 W halogen lamp at a distance of 3.0 cm with a Toshiba L-39 cut-off filter at 30 °C. The $MV^{\bullet+}$ concentration was determined by the absorbance at 605 nm using the molar coefficient 13,000 M^{-1} cm^{-1}.

2.4. Ethanol production

A sample solution containing Zn Chl-e_6 (100 μM), MV^{2+}, NADPH (3.3 mM), AldDH (0.22 μM), ADH (6.7 nM) and sodium acetate (30 mM) in 50 mM sodium pyrophosphate buffer (pH 7.4) was deaerated by freeze–pump–thaw cycles repeated 6 times and then flushed with nitrogen gas for 5 min, and then irradiated with a 250 W halogen lamp at a distance of 3.0 cm with a Toshiba L-39 cut-off filter at 30 °C. The produced ethanol was measured by gas chromatography using a Sorbitol 25%-Gasport B column (2 m × 3 mm i.d., GL Sciences) attached to a Schimadzu GC-8A gas chromatograph (oven temperature, 100 °C; carrier gas, N_2; flow rate, 21.8 ml min^{-1}).

3. Results and discussion

3.1. Photoreduction of MV^{2+}

Photoreduction of MV^{2+} is one of the important step in photochemical synthesis of ethanol from acetate system. To attain the highest yield of reduced MV^{2+} ($MV^{\bullet+}$), the reaction conditions of photoreduction of MV^{2+} consisting of NADPH and Zn Chl-e_6 were investigated. MV^{2+} concentrations in the solution were varied between 0.12 and 12 mM. When the sample solution was irradiated, the accumulation of $MV^{\bullet+}$ was observed in all cases. The rate of $MV^{\bullet+}$ formation increased with increasing MV^{2+} concentration. Generally, alcohols, methanol, ethanol and so on are used as an electron donor in semiconductor-based photocatalytic systems for hydrogen production. Therefore, photoreduction of MV^{2+} consisting of Zn Chl-e_6 (100 μM), MV^{2+}(1.2 mM), and ethanol (3.3 mM) in place of NADPH in 3.0 ml of 50 mM sodium pyrophosphate buffer (pH 7.4) was tested with visible-light irradiation. No $MV^{\bullet+}$ formation was observed in this reaction condition, indicating that ethanol was not act as an electron donor in this photoredox system.

3.2. Ethanol production from acetate with photoredox system and dehydrogenases

As the MV^{2+} photoreduction system with the photosensitization of Zn Chl-e_6 was developed, the photochemical ethanol synthesis from acetate was attempted above condition with AldDH and ADH. When the sample solution was irradiated with a 250 W halogen lamp, ethanol production is shown in Fig. 4. The ethanol was produced with irradiation time and ethanol concentration produced was estimated to be 1.4 mM after 150 min irradiation. In contrast, no ethanol production was observed under dark condition (open square in Fig. 4). Moreover, no ethanol production was

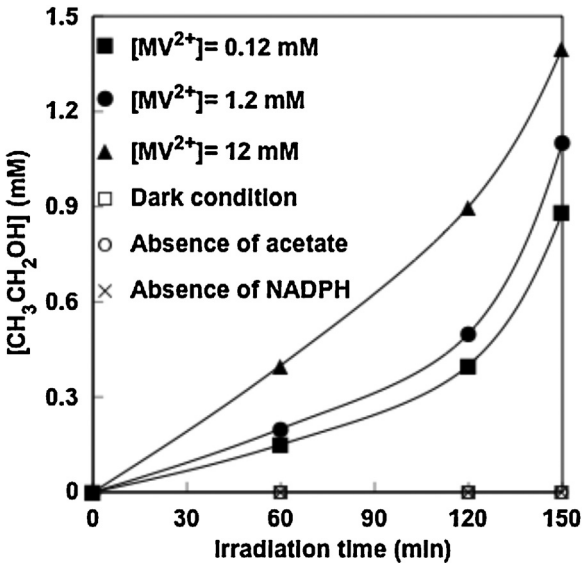

Fig. 4. Time dependence of photochemical ethanol synthesis under steady state irradiation with visible light using a 250 W halogen lamp at a distance of 3.0 cm. The solution contained Zn Chl-e_6 (100 μM), MV^{2+}, NADPH (3.3 mM), AldDH (0.22 μM), ADH (6.7 nM) and sodium acetate (30 mM) in 50 mM sodium pyrophosphate buffer (pH 7.4). Closed square, circle and triangle are 0.12, 1.2 and 12 mM of MV^{2+}, respectively. Open square, circle and X are dark, absence of acetate and absence of NADPH conditions, respectively. The reaction temperature is 30 °C.

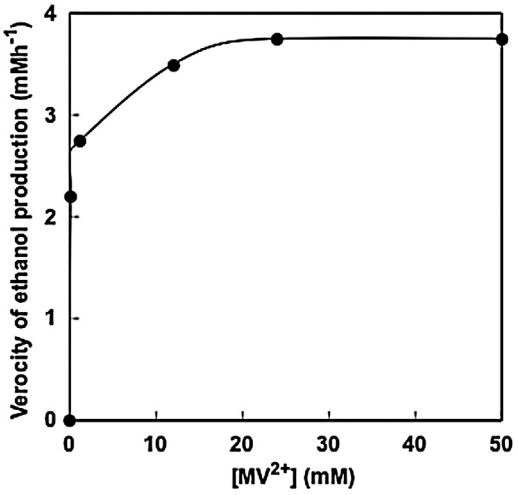

Fig. 5. The relationship between MV^{2+} concentration and the velocity of ethanol production in the system consisting of Zn Chl-e_6 (100 μM), MV^{2+}, NADPH (3.3 mM), AldDH (0.22 μM), ADH (6.7 nM) and sodium acetate (30 mM) in 50 mM sodium pyrophosphate buffer (pH 7.4).

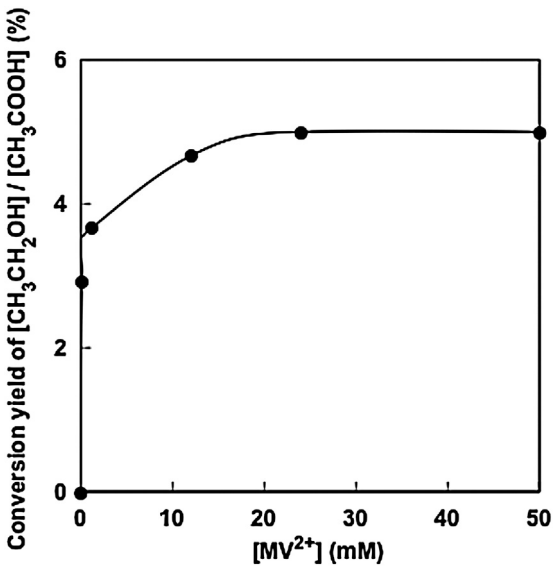

Fig. 6. The relationship between MV^{2+} concentration and the conversion yield of acetate to ethanol in the system consisting of Zn Chl-e_6 (100 μM), MV^{2+}, NADPH (3.3 mM), AldDH (0.22 μM), ADH (6.7 nM) and sodium acetate (30 mM) in 50 mM sodium pyrophosphate buffer (pH 7.4).

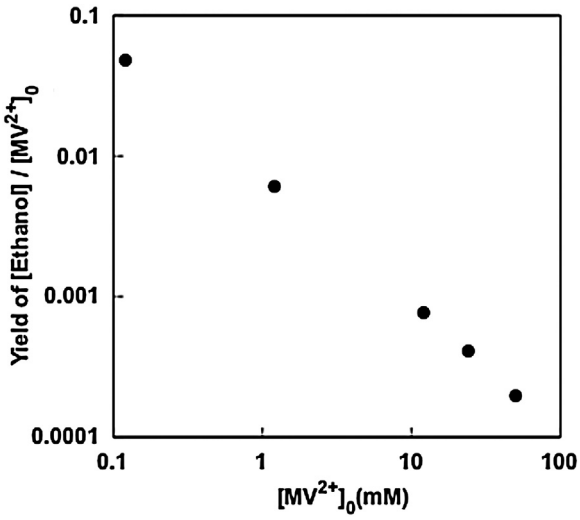

Fig. 7. The relationship between initial MV^{2+} concentration ($[MV^{2+}]_0$) and the conversion yield of concentration of ethanol produced after 150 min irradiation to initial MV^{2+} concentration ([Ethanol]/$[MV^{2+}]_0$) for ethanol production in the system consisting of Zn Chl-e_6 (100 μM), MV^{2+}, NADPH (3.3 mM), AldDH (0.22 μM), ADH (6.7 nM) and sodium acetate (30 mM) in 50 mM sodium pyrophosphate buffer (pH 7.4).

also observed in the absence of acetate (open circle) or NADPH (X). These results indicate that the photochemical synthesis of ethanol from acetate with AldDH and ADH via the photoreduction of MV^{2+} using Zn Chl-e_6 photosensitization as shown in process 2 of Fig. 3.

As shown in Fig. 4, concentration of produced ethanol increased with increasing MV^{2+} concentration in this system. Fig. 5 shows the relationship between the MV^{2+} concentration and the ethanol production velocity. The velocity value was determined by ethanol concentration after irradiation for 150 min. The ethanol concentration increased with MV^{2+} concentration up to 25 mM and then reached constant value of 4.6 m Mh^{-1}. Fig. 6 shows the relationship between the MV^{2+} concentration and the conversion yield of acetate to ethanol after irradiation for 150 min. The conversion yield of acetate to ethanol also increased with MV^{2+} concentration up to 25 mM and then reached constant value of 5%. Since the ethanol production rate and the conversion yield of acetate to ethanol were constant with the increase of MV^{2+} concentration, the of ethanol production was dependent on the efficiency of the $MV^{\bullet+}$ production. So, the concentration of ethanol produced after 150 min irradiation on the initial concentration of MV^{2+} was studied. Fig. 7 shows the relationship between the initial concentration of MV^{2+} ($[MV^{2+}]_0$) and conversion yield of MV^{2+} to ethanol. The conversion yield of MV^{2+} to ethanol was decreased with increasing the initial concentration of MV^{2+} concentration. We previous reported that the efficiency of the $MV^{\bullet+}$ production with visible-light sensitization of water-soluble zinc porphyrin was decreased under higher concentration of MV^{2+} [17]. Thus, this result suggests that ethanol production is dependent on the efficiency of the $MV^{\bullet+}$ production with visible-light sensitization of Zn Chl-e_6.

To improve the ethanol synthesis from acetate in this system, thus, it is necessary to develop the artificial coenzyme based on the viologen skeleton with highly affinity for AldDH and ADH.

4. Conclusion

As the largest single use of ethanol is as a motor fuel and fuel additive, we devoted to the visible light induced synthesis of ethanol from carbon dioxide, natural gas such as methane and water. One of the important steps is the photochemical ethanol synthesis from acetate using photoredox and catalyst system. The development of the photochemical ethanol synthesis from acetic acid with AldDH and ADH, and reduced MV^{2+} produced by the visible light photosensitization of ZnChl-e_6 in the presence of NADPH as an electron donor is studied. The concentration of ethanol produced was 1.4 mM after 150 min irradiation under the condition of NADPH (3.3 mM), ZnChl-e_6 (100 μM), MV^{2+} (12 mM), AldDH (0.22 μM), ADH (6.7 nM) and sodium acetate (30 mM) in 50 mM of sodium pyrophosphate buffer pH 7.4). The conversion yield of acetate to ethanol was reached to 5% after 150 min irradiation.

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